VIM-4 carbapenemase-producing *Enterobacter cloacae* in the United Arab Emirates

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Abstract

Screening 34 carbapenem non-susceptible Enterobacteriaceae recovered in Abu Dhabi hospitals identified an *Enterobacter cloacae* strain carrying bla_{VIM-4}, bla_{CMY-4} and $bla_{CTX-M-15}$. It was isolated from the urine of an Egyptian patient repeatedly hospitalized and treated with broad-spectrum antibiotics, including carbapenems, in the United Arab Emirates. The bla_{VIM-4} coding class 1 integron, highly similar to In*416*, was carried on a 175-kilobase non-conjugative incA/C type plasmid also hybridizing with the bla_{CMY-4} probe. This is the first detailed report on the isolation of a Verona integron-encoded metallo-β-lactamase (VIM) -producing enteric bacterium in the Arabian Peninsula with characteristics suggestive of spreading from the Mediterranean region.

Keywords: carbapenemase, *Enterobacter*, Middle East, multi-drug resistance, VIM-4

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Enteric bacteria producing acquired carbapenemases have been increasingly reported worldwide. Verona integron-encoded metallo-β-lactamase (VIM) -producing Enterobacteriaceae have been isolated in several countries with high prevalence noted in the Mediterranean region [1]. The expression of bla_{VIM} genes confers resistance to all β-lactams except aztreonam. The single amino acid variant of the first reported VIM-1 enzyme, VIM-4 was described in a *Pseudomonas aeruginosa* strain isolated in Greece in 2002 [2]. Subsequently, VIM-4 has been identified in *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Acinetobacter* spp., *Aeromonas hydrophyla* and *Escherichia coli* [3–9]. VIM-4-producing Enterobacteriaceae strains reportedly also harboured various other β-lactamases, e.g. KPC-2 [6], CMY-4 [10,11], CTX-M-15 [8,10], SHV-12 [3], SHV-2a [4] and TEM-1 [10], respectively. The bla_{VIM-4} gene has been described exclusively as part of a class 1 integron located, in most of the cases, on conjugative plasmids of varying size, which, if tested, all belonged to the A/C incompatibility group (summarized in Table 1).

The VIM-type metallo-β-lactamase-producing bacteria are rarely described from the Arabian Peninsula. *Acinetobacter baumannii* and *P. aeruginosa* producing VIM-2 were reported from Kuwait and Saudi Arabia [12,13]. However, at the time of writing, a search of the literature did not reveal any description of VIM-producing Enterobacteriaceae from the Gulf region, beyond a conference abstract from Kuwait [14]. Here we report on the isolation and first detailed analysis of a VIM-producing enteric bacterium from the Arabian Peninsula, an *Enterobacter cloacae* strain carrying the bla_{VIM-4} gene.

Screening 34 carbapenem non-susceptible clinical Enterobacteriaceae strains isolated in Abu Dhabi Emirate by a multiplex PCR [15] identified known carbapenemase genes in 22 isolates. bla_{NDM} was found in six *K. pneumoniae*, one *E. cloacae*, two *Escherichia coli* and one *Citrobacter freundii*, while $bla_{OXA-48-like}$ was identified in eight *K. pneumoniae* and three *Escherichia coli* strains, respectively. An *E. cloacae* strain positive for bla_{VIM} (ABC104) was also identified. This strain was isolated in January 2012 from the urine sample of a 45-year-old Egyptian male, permanent resident of the United Arab Emirates. The man had a spinal cord injury and urinary incontinence since the age of 12 years. Two years before sampling the patient had stayed in Egypt for a short while on family business, but he had never received any medical treatment beyond the United Arab Emirates. He was diagnosed with lumbosacral ependymoma in 2009 and underwent subtotal excision of the mass in June 2011. Because of residual tumour, the surgical wound never healed and was secondarily infected. Superficial wound cultures grew *K. pneumoniae*, methicillin-resistant *Staphylococcus aureus* and normal skin flora. Over the ensuing months, he was treated with multiple courses of intravenous and oral antibiotics including piperacillin–tazobactam, ertapenem, meropenem, amoxicillin–clavul-

TABLE 1. Genotypic characteristics of VIM-4 producing Enterobacteriaceae

Species	Country	GenBank accession no.	Class I integron structure							VIM plasmid		Reference	RT
			Gene cassettes (GC)					3′ end		Size	Co-harboured β-lactamases[a]		
			GC1	GC2	GC3	GC4	GC5						
Enterobacter cloacae	UAE	JX275775	bla_{VIM-4}	*aacA7*	*dfrA1*	*ΔaadA1*	*smr*	*ISPa21*	*qacEΔ1* *sul1*	175 kb	incA/C	**CMY-4, TEM-1,** CTX-M-15	This study
Escherichia coli	Russia	Unknown	bla_{VIM-4}	*aacA7*	*dfrA1*	*ΔaadA1*	*smr*	*ISPa21*		40 kb	incA/C	CTX-M-15	[8]
E. cloacae, Klebsiella pneumoniae	Italy	AJ704863	bla_{VIM-4}	*aacA7*	*dfrA1*	*ΔaadA1*	*smr*	*ISPa21*		Unknown	incA/C	**CMY-4**	[11]
K. pneumoniae	Tunisia	AM181293	bla_{VIM-4}	*aacA7*	*dfrA1*	*ΔaadA1*	ND	ND		>130 kb	NT	**CMY-4,** TEM-1, CTX-M-15	[10]
K. pneumoniae, Klebsiella oxytoca	Hungary	GU181265, GU181269	*aacA4*	bla_{VIM-4}	–	–	–	*qacEΔ1*	*sul1*	90 kb	NT	None	[7]
E. cloacae	Greece	EF467306	bla_{VIM-4}	*aacA7*	*dfrA1*	*aadA1*	–	*qacEΔ1*		40 kb	NT	**SHV-2a**	[4]
K. pneumoniae	Greece	unknown	bla_{VIM-4}	–	–	–	–	–		175 kb	NT	CMY-4, KPC-2	[6]

kb, kilobase; ND, not detected; NT, not tested; RT, replicon type; VIM-4, Verona integron-encoded metallo-β-lactamase 4.
[a]Beta-lactamases shown in bold are carried on the VIM plasmids.

anate and levofloxacin for a cumulative total of 138 Defined Daily Doses. In January 2012, he was admitted with back pain and cloudy urine.

The ABC104 strain was resistant to imipenem (MIC = 16 mg/L), meropenem (MIC = 8 mg/L), ertapenem (MIC = 4 mg/L), ceftazidime (MIC >128 mg/L), cefotaxim (MIC >128 mg/L), cefepime (MIC = 64 mg/L), aztreonam (MIC >128 mg/L), cefoperazone (MIC = 64 mg/L), trimethoprim/sulphamethoxazole (MIC = 8/76 mg/L), chloramphenicol (MIC = 32 mg/L), gentamicin (MIC = 32 mg/L), tobramycin (MIC = 48 mg/L), netilmicin (MIC = 32 mg/L), ciprofloxacin (MIC = 32 mg/L), moxifloxacin (MIC = 12 mg/L), levofloxacin (MIC = 12 mg/L), tetracycline (MIC = 64 mg/L) and minocycline (MIC = 32 mg/L) and exhibited sensitivity to amikacin (MIC = 6 mg/L) and colistin (MIC = 0.125 mg/L), only as tested by microdilution according to the CLSI standards [16]. It carried the bla_{VIM-4} gene on an incA/C type [17] plasmid of c.175 kilobases as detected by Southern blotting of the S1-digested genomic DNA (Fig. 1). Attempts to conjugally transfer the plasmid into an azid-resistant derivative of *Escherichia coli* J53 failed at 30°C, as well as at 37°C. By PCR mapping and sequencing using primers designed based on

FIG. 1. Southern blot of *Enterobacter cloacae* strain ABC104 S1-digested genomic DNA. Gel, left lane: λ concatamer. Oligonucleotide probes of the respective genes (see above the membrane strips) were PCR-generated and labelled with a DIG-DNA labelling kit (Roche Diagnostics GmbH, Mannheim, Germany). After hybridization and colour development the probe was removed and the membrane was re-hybridized with the next probe. Detection was achieved by an alkaline-phosphatase-based reaction according to the manufacturer's instructions.

previously published sequences (GenBank accession numbers. AJ704863 and AY339625) the bla_{VIM-4} gene was identified as part of a class 1 integron (Table 1). The integron was similar to other bla_{VIM-4}-containing integrons identified in various Enterobacteriaceae isolates in Italy, Russia, Tunisia and Greece (Table 1) [4,8,10,11]. However, unlike in the Italian isolate VA-416/02 [11], in ABC104 the conserved 3′ end of the class 1 integron was identified downstream of the IS*Pa21* (GenBank accession number JX27577). ABC104 also harboured other β-lactamase genes: $bla_{CTX-M-15}$, bla_{TEM-1} and bla_{CMY-4}; bla_{CMY-4} being localized on the same plasmid as *bla*VIM-4 by Southern blotting. The $bla_{CTX-M-15}$ gene was carried on a plasmid of *c.*300 kilobases, whereas a probe for bla_{TEM-1} hybridized with both plasmids (Fig. 1).

This first description of a VIM-4-producing enteric bacterium in the Arabian Peninsula adds a further resistance mechanism to the list of carbapenemases, mostly of the NDM and OXA-types, in Enterobacteriaceae already reported from the region [18–20]. The nationality and travel history of the patient, and the fact that the other β-lactamases, the plasmid, and the class 1 integron containing the bla_{VIM-4} of ABC104 were all highly similar to those previously described from Italy [11] and North Africa [10] suggests the possibility of these resistance genes, or the organism itself, spreading from the Mediterranean region to the Gulf.

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Transparency Declaration

No competing financial interests exist.

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